Pharmacoinformatics: Equations for Serum Drug Assay Error Patterns; Implications for Therapeutic Drug Monitoring and Dosage.

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ABSTRACT

Pharmacoinformatics is the area of Medical Informatics concerned with modeling and simulation of the behavior of drugs, and control of such behavior by individualized dosage regimens for each patient to achieve explicitly chosen therapeutic goals. The credibility of serum concentration data is a major factor in such modeling.

The present report examines a more precise way of describing the credibility of such data with a collection of polynomial equations, developed from routine survey data of the College of American Pathologists, which improve the description of the credibility of serum level results when compared to the usual practice of describing the assay coefficient of variation in the usual manner and then not using such information either in population pharmacokinetic modeling or in actual therapeutic drug monitoring. These equations can be used until each laboratory can develop its own assay error patterns with its own similar polynomial equations.

INTRODUCTION

In Medical Informatics and its branch of Pharmacoinformatics, Bayesian methods to make individualized pharmacokinetic models of drug behavior in patients have led to improved prediction (and therefore control) of future serum drug concentrations [1]. They use a combination of population pharmacokinetic parameter values and the patient's own measured serum concentrations.. The Bayesian posterior parameter values are found by minimizing the Bayesian objective function [2]:

$$[\text{SUM}\ \frac{(Ppop - Ppt)^2}{SD^2\ Ppop} + \text{SUM}\ \frac{(Cobs - Cpt)^2}{SD^2\ Cobs}]$$

where Ppop and Ppt represent the parameter values of the population pharmacokinetic model and of the patient's individualized model respectively, Cobs and Cpt represent the observed serum drug concentrations and the estimates of those concentrations made with the patient's own individualized pharmacokinetic model respectively, and SD Ppop and SD Cobs are the standard deviations of the various population parameter values and of the various observed serum concentrations respectively.

As shown in this objective function, the credibility of each population pharmacokinetic parameter value is determined by the reciprocal of the variance (the square of the SD) which it has been found to have. Thus the SD of each population parameter value leads directly to the correct index of credibility for each population parameter value.

The same is true for the data of the measured serum drug concentrations. However, most laboratories have simply made sure that the SD's of each assay are within acceptable limits. Once this is done, the actual error has usually been ignored for purposes of therapeutic drug monitoring.

Practical Determination of Serum Assay Error Patterns

What is needed is a practical means to determine the standard deviation of each serum drug concentration as it is routinely measured by the clinical laboratory. A convenient and practical way to do this is to do replicate measurements of representative samples (at least in quadruplicate) and to determine the mean and SD of each sample. This can be done, for example, on a blank, a low, an intermediate, a high, and a very high sample, so that the entire assay range is covered [3]. One can fit the relationship between measured concentration and SD with a polynomial equation, usually of second order. Using this equation, it is easy to calculate the probable SD with which any subsequent single serum concentration is measured within that range. For example, at the suggestion of Gilman [4] the error pattern of the EMIT gentamicin assay in use at the Los Angeles County-USC Medical Center was determined, and its polynomial equation was found to be:

$$SD\ (ug/ml) = 0.56708 - 0.10563C + 0.016801C^2$$

A graph of this very heteroschedastic relationship is shown in Figure 1.

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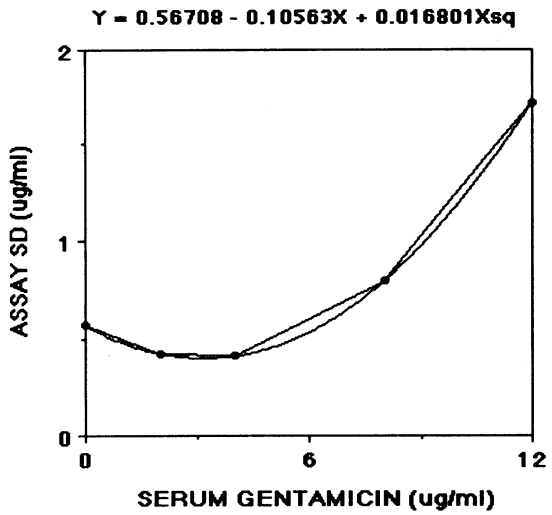

Figure 1. *Graph of relationship between concentration and assay SD for the EMIT Gentamicin assay.*

The coefficients of the polynomial equation are then stored with the Gentamicin population model in the USC*PACK clinical program [5] for adaptive control of Gentamicin dosage regimens for correct weighting of serum concentrations.

ANALYSIS OF THE COLLEGE OF AMERICAN PATHOLOGISTS SURVEY

The College of American Pathologists (CAP) sends out specimens containing stated drug concentrations to clinical laboratories which report their findings back to the CAP. The CAP then reports the means and SD's of these findings, and the number of laboratories reporting. We examined the results published in CAP Data Sets 1987 ZM-D, 1988 Z-D, 1989 Z-B, Z-C, and Z-D, and 1990 Z-A, Z-B, and Z-C, for Amikacin, Gentamicin, Digoxin, Lidocaine, Theophylline, and Vancomycin. We took the means and SD's of the results found for the various stated specimens and fitted them with a polynomial, usually of second order, to provide a library of error patterns for the above assays. R^2, the square of the correlation coefficient between concentration and SD, represents the proportion of the variation between them which is explained by the fitted polynomial [6]. A value approaching 1.0 reflects little scatter of the data, while a low R^2 reflects considerable scatter, and an assay which is less consistent in its errors over its range [6].

RESULTS

Amikacin

Fifteen sample means, ranging from 1.1 to 30.0 ug/ml, and their SD's, obtained from 339 to 725 reporting laboratories, provided the data. The following polynomial equations for the various assay error patterns were found.

Abbott TDx SD (ug/ml) = 0.30156 + 0.0053855C + 0.0011184 C^2, R^2 = 0.983
Dupont ACA SD (ug/ml) = 0.46475 + 0.0281310C + 0.0021305C^2, R^2 = 0.939
Syva Emit SD (ug/ml) = 0.23237 + 0.0470150C + 0.0016876C^2, R^2 = 0.965
All Methods SD (ug/ml) = 0.32272 + 0.0183650C + 0.0012051C^2, R^2 = 0.983

The Abbott TDx assay was the most precise.

Gentamicin

Seventeen sample means, ranging from 0.9 to 17.8 ug/ml, and their SD's, obtained from 2512 to 3600 reporting laboratories provided the data. The following polynomial equations for the various assay error patterns were found.

Abbott TDx SD (ug/ml) = 0.02458 + 0.04948C + 0.0020318C^2, R^2 = 0.957
Dupont ACA SD (ug/ml) = 0.25719 - 0.016215C + 0.0081998C^2, R^2 = 0.982
Syva Emit SD (ug/ml) = 0.14078 - 0.002263C + 0.0184060C^2, R^2 = 0.991
All Methods SD (ug/ml) = 0.09114 - 0.043524C + 0.0045964C^2, R^2 = 0.992

The Abbott TDx assay was the most precise.

Digoxin

Seventeen sample means ranging from 0.2 to 3.0 ng/ml, and their SD's, obtained from 3160 to 4454 reporting laboratories provided the data. The following polynomial equations were found.

Abbott TDx SD (ng/ml) = 0.09211 + 0.0088626C + 0.0099406C^2, R^2 = 0.948
Baxter Stratus SD (ng/ml) = 0.144211 - 0.048708C + 0.022917C^2, R^2 = 0.911
ClinicalAssays SD (ng/ml) = 0.086719 + 0.017052C + 0.011857C^2, R^2 = 0.881
Dupont ACA SD (ng/ml) = 0.15560 - 0.056293C + 0.035574C^2, R^2 = 0.562
Syva Emit SD (ng/ml) = 0.16111 + 0.051579C, R^2 = 0.451
All Methods SD (ng/ml) = 0.12312 - 0.0073104C + 0.020257C^2, R^2 = 0.951

As shown in Figure 2, the Abbott TDx assay was the most precise, and also had the highest R^2 (coefficient of the determination). Because of this, the error pattern of that assay is well characterized by such a polynomial equation.

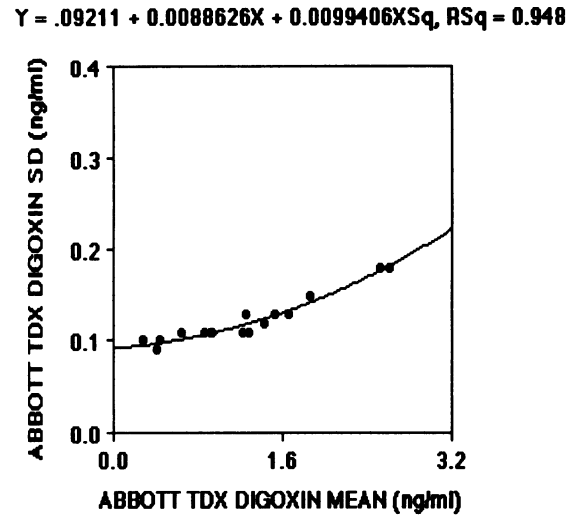

Figure 2. *Graph of relationship between concentration and assay SD for the Abbott TDx digoxin assay.*

On the other hand, when the Syva Emit assay findings were fitted with a second order polynomial, the curve reached a peak and then began to bend downward. This could yield dangerously low estimates of the SD when extrapolated beyond the range reported here (0.2 to 3.0 ng/ml). Because of this, and because the first order polynomial had essentially the same value of R^2, the first order equation was used here, as shown in Figure 3. The Syva Emit and Dupont ACA assays had the lowest values of R^2, showing that their error pattern had great scatter, while the Abbott TDx had a high value of R^2, showing that its error pattern had little scatter. The Abbott TDx assay was the most precise.

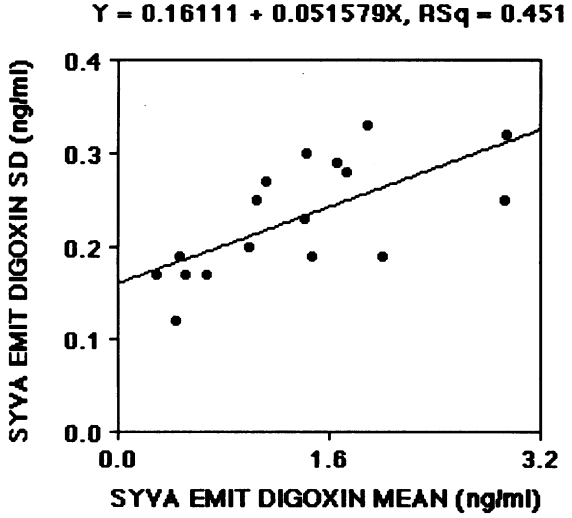

Figure 3. *Graph of relationship between concentration and assay SD for the Syva EMIT Digoxin assay.*

Lidocaine

Fifteen sample means ranging from 0.3 to 6.0 ug/ml, and their SD's, obtained from 430 to 799 reporting laboratories, provided data. The following polynomial equations were obtained.

Abbott TDx SD (ug/ml) = 0.053404 + 0.020234C + 0.0036386C^2, R^2 = 0.971
Dupont ACA SD (ug/ml) = 0.319570 - 0.132040C + 0.0265960C^2, R^2 = 0.407
Syva Emit SD (ug/ml) = 0.158580 - 0.013422C + 0.0126140C^2, R^2 = 0.924
All Methods SD (ug/ml) = 0.083569 + 0.008491C + 0.0068741C^2, R^2 = 0.985

The Dupont ACA assay, as shown by its low value of R^2, had a widely varying and inconsistent SD, while the Abbott TDx and Syva Emit assay SD's were consistent. The Abbott TDx assay was the most precise.

Theophylline

Seventeen sample means ranging from 3.0 to 30.0 ug/ml, and their SD's, obtained from 3682 to 4696 reporting laboratories, provided data. The following polynomial equations were obtained.

Abbott TDx SD (ug/ml) = 0.22605 + 0.023955C + 0.00056926C^2, R^2 = 0.978
Baxter Stratus SD (ug/ml) = 0.07889 + 0.083394C, R^2 = 0.985
Dupont ACA SD (ug/ml) = 0.29967 + 0.010201C + 0.1379800C^2, R^2 = 0.963
HPLC assay SD (ug/ml) = 1.04060 - 0.120450C + 0.0093092C^2, R^2 = 0.707
Syva Emit SD (ug/ml) = 0.21770 + 0.057018C + 0.07131800C^2, R^2 = 0.972
All Methods SD (ug/ml) = 0.25463 + 0.039573C + 0.00088179C^2, R^2 = 0.976

The Abbott TDx assay was the most precise, The HPLC assay was the least. The Dupont ACA assay was next most precise. The HPLC polynomial had the lowest value of R^2 and the greatest scatter.

Vancomycin

Fifteen sample means ranging from 4.9 to 40.0 ug/ml, and their SD's, obtained from 645 to 862 reporting laboratories, provided data. The following polynomial equations were obtained.

Abbott TDx SD (ug/ml) = 0.57694 + 0.012816C + 0.00058286C^2, R^2 = 0.971
Syva Emit SD (ug/ml) = 0.93214 + 0.023689C + 0.00177690C^2, R^2 = 0.971

All Methods SD (ug/ml) = 0.59421 + 0.012291C + 0.00071299C^2, R^2 = 0.979

Both error patterns were well characterized by their equations, with R^2 values over 0.97. The Abbott TDx assay was the more precise.

DISCUSSION

Sources of Error

The errors reported by the College survey are a mixture of within - run and between - run laboratory errors, as well as within - laboratory and between - laboratory errors. The more laboratories reporting, the more closely each sample SD reported is to the true overall SD of the assay and the interlaboratory variation.

The Importance of Models

Recently, a change is taking place in the process by therapeutic drug monitoring and the individualization of drug dosage regimens is performed. Less attention is being paid to the interpretation of the raw data of the individual serum concentration results, and more is being paid to the pharmacokinetic or pharmacodynamic model fitted to such data. A patient's clinical behavior may not correlate with his serum levels, while correlation of his clinical behavior with the behavior of his fitted model is often good and very revealing, especially when the clinical effect of the drug correlates better with concentrations in the peripheral nonserum compartment, as with digoxin [7]. Use of models containing nonserum compartments is providing new views of the kinetic behavior of many drugs, including the aminoglycosides, lidocaine, digoxin, digitoxin, and vancomycin. Proper Bayesian fitting, using the correct assay error pattern, is essential. Inaccurate assay error patterns or simple assumptions of a certain coefficient of variation can lead to grossly inaccurate model parameter values, both in individually fitted patient pharmacokinetic models and in population pharmacokinetic modeling [8].

The Importance of Measuring Blanks

It is interesting that in none of the samples sent out by the College was there a blank sample. Clinical laboratories, however, usually characterize the sensitivity of their assays by choosing a value two SD's above a blank. When concentrations lower than those clearly detectable are encountered, they are often simply reported as being "less than X", where X is two SD's above the blank.

The Importance of Reporting Low Concentrations Below Detectable Limits

While the above practice is useful in toxicological analysis in making a firm decision as to whether a substance is present in the body or not, it is a distinct obstacle to optimal therapeutic drug monitoring. In therapeutic drug monitoring there is no question that the drug has been given. One knows this from the history, the orders, and the nurses' notes, for example. Indeed, many clinical laboratories will not measure a serum drug concentration unless the time since the last dose is stated on the request slip. Since the patient never excretes the last molecule of the drug, there is no question that the drug is still present in the body. The only question is its concentration. Low trough aminoglycoside concentrations for example, below those clearly detectable, are not only useful but necessary for therapeutic drug monitoring and Bayesian pharmacokinetic modeling. To withhold such results renders that measurement useless for Bayesian modeling. A vital data point is absent.

Rather than reporting a Gentamicin concentration as "less than 0.5ug/ml" for example, the laboratory can easily report the actual value found, and can report it as "0.1 ug/ml, below the secure detectable limits of 0.5 ug/ml", for example. This procedure will answer both the needs of the toxicologists and the pharmacokineticists.

The Importance of Collecting High Serum Concentrations

The CAP Survey paid most attention to determining errors for concentrations within the therapeutic range. However, low trough concentratio are frequently found.

On the other hand, it is equally important to know know the errors of concentrations found well into the toxic range. Because of this, when either low or high concentrations are encountered, one might suggest that the laboratory run them in replicate as many times as possible, especially to better characterize the error of the assay at its high end, and to extend the range of the known assay error.

The Importance of Improving Assay Precision at the High End

When doing Bayesian fitting, one can only give equal weight to various serum concentrations when they are homoschedastic and have the same SD. This may be an unrealizable ideal.

In contrast, a heteroschedastic assay error pattern is one in which the assay SD changes over its working range. Even an assay with a constant coefficient of variation is very heteroschedastic. If one assumes a constant coefficient of variation, a concentration of 1.0 ug/ml, for example, has a weight 100 times greater than that of a concentration of 10.0 ug/ml, and a concentration of 0.1 ug/ml has a weight 100 times that of the concentration of 1.0 ug/ml, and 1000 times that of the concentration of 10.0 ug/ml! Because of this, when a constant coefficient of variation is assumed for an assay used in Bayesian fitting, high concentrations will be relatively ignored compared to lower ones, and the fitted model will not approach the high concentrations as closely as one might wish. This is also true for the polynomial equations described above. The difference here is that the polynomial equations are based on carefully measured SD's for each assay.

One of two things needs to be improved. Either the current Bayesian fitting procedure based on weighting of the data points by the reciprocal of their variance (Fisher information) is incorrect, or the assays need to have their precision improved at the high end to make them more homoschedastic. To discard such weighting and the concept of Fisher information [9] would be to overthrow several decades of carefully acquired and searchingly criticized mathematical and statistical knowledge. To improve the precision of assays at their high end is probably the most constructive thing to do. It may even be possible, for example, to alter the ratios of reagents so that the ratio of bound and unbound drug in the assay can be changed, with a resultant change in the error pattern toward homoschedasticity.

CONCLUSION

The results of the survey by the College of American Pathologists have been analyzed to provide a library of polynomial equations which characterize the error patterns of several assays over their working range. These equations can be used to improve the precision of Bayesian fitting of pharmacokinetic models until each laboratory can determine its own error patterns for the drugs it monitors. The procedure is easy to do, is cost-effective, and can be repeated as desired from time to time. Some suggestions are made for improving the quality of therapeutic drug monitoring and for subsequent surveys.

Acknowledgements

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